Higher net acid excretion is associated with a lower risk of kidney disease progression in patients with diabetes

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Higher diet-dependent nonvolatile acid load is associated with faster chronic kidney disease (CKD) progression, but most studies have used estimated acid load or measured only components of the gold standard, net acid excretion (NAE). Here we measured NAE as the sum of urine ammonium and titratable acidity in 24-hour urines from a random subset of 980 participants in the Chronic Renal Insufficiency Cohort (CRIC) Study. In multivariable models accounting for demographics, comorbidity and kidney function, higher NAE was significantly associated with lower serum bicarbonate (0.17 mEq/l lower serum bicarbonate per 10 mEq/day higher NAE), consistent with a larger acid load. Over a median of 6 years of follow-up, higher NAE was independently associated with a significantly lower risk of the composite of end-stage renal disease or halving of estimated glomerular filtration rate among diabetics (hazard ratio 0.88 per 10 mEq/day higher NAE), but not those without diabetes (hazard ratio 1.04 per 10 mEq/day higher NAE). For comparison, we estimated the nonvolatile acid load as net endogenous acid production using self-reported food frequency questionnaires from 2848 patients and dietary urine biomarkers from 3385 patients. Higher net endogenous acid production based on biomarkers (urea nitrogen and potassium) was modestly associated with faster CKD progression consistent with prior reports, but only among those without diabetes. Results from the food frequency questionnaires were not associated with CKD progression in any group. Thus, disparate results obtained from analyses of nonvolatile acid load directly measured as NAE and estimated from diet suggest a novel hypothesis that the risk of CKD progression related to low NAE or acid load may be due to diet-independent changes in acid production in diabetes.

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Metabolic acidosis is a modifiable risk factor for progression of chronic kidney disease (CKD).[1–3] Treatment of CKD patients with sodium bicarbonate slowed progression in small randomized studies,[4,5] but it remains unclear whether sodium bicarbonate exerts its beneficial effects by increasing the systemic pH or by lowering the amount of acid that must be excreted in the urine, also known as the nonvolatile acid load.[6] Previous studies suggest that higher nonvolatile acid load is associated with progression of CKD; however, those studies relied on *estimated* acid load from dietary data rather than direct measurements, and many focused exclusively on patients with hypertensive kidney disease.[7–9] Because patients with diabetes exhibit differences in urinary acidification and acid production compared with those without diabetes,[10] the clinical impact of acid load on CKD progression may also differ in patients with diabetic nephropathy compared with other forms of CKD.

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Excreting the load of nonvolatile acids generated during metabolism is a critical homeostatic function of the kidneys. Nonvolatile acids (i.e., H^+) are produced when (i) sulfur-containing amino acids are oxidized to inorganic sulfate and (ii) the conjugate base of endogenously produced organic acids (e.g., citric acid) are excreted in the urine as an organic anion salt (e.g., sodium citrate).[11–13] Alkali may be ingested in the form of alkali supplements or metabolizable organic anion salts found abundantly in fruits and vegetables, both of which may buffer nonvolatile acids, at least in part.[9,14,15] Thus, the net load of nonvolatile acid that must be excreted by the kidney equals the difference between acids produced and alkali consumed in foods or supplements. The kidney excretes the nonvolatile acid load as ammonium (NH_4^+) or as titratable acid bound to anionic urinary buffers such as phosphate and creatinine.[16,17] Regulation of acid excretion and production maintains acid-base homeostasis in response to dietary changes or systemic acid-base perturbations.[18,19]

Because the daily net acid excretion approximates the daily nonvolatile acid load in the steady state, direct measurement of 24-hour urinary net acid excretion is the reference standard for measuring acid load.[20] However, the relationship between 24-hour urine net acid excretion and renal outcomes in patients with CKD remains unknown. We hypothesized *a priori* that greater 24-hour net acid excretion would be associated with a higher risk of CKD progression overall, particularly among those with diabetes because they have a greater predisposition to metabolic acidosis.[21] To test this hypothesis, we directly measured net acid excretion in participants from the Chronic Renal Insufficiency Cohort (CRIC) Study, a diverse CKD population including approximately equal numbers of patients with and without diabetes. Additionally, previous investigations questioned whether nonvolatile acid load truly equals acid excretion in the setting of CKD.[22–24] Thus, for comparison, we also estimated nonvolatile acid load from self-reported dietary intake data similar to previous reports.[15]

RESULTS

Physiology of acid production and excretion in CKD

We measured acid excretion in baseline 24-hour urine collections obtained in a randomly selected sample of 1000 participants from the CRIC Study. We calculated net acid excretion as the sum of urinary ammonium and titratable acidity, calculated from urinary pH, phosphorus, and creatinine. Nineteen urine samples with a pH ≥7.4 were excluded from further analysis due to concern for bacterial overgrowth. The ratio of urinary ammonium to urinary sulfate, a biomarker of acids produced from metabolism of sulfur-containing amino acids, was dramatically increased in these specimens (median 3.1 vs. 0.6 in urine samples with a pH ≥7.4 compared with <7.4; $P < 0.01$), suggesting exogenous ammonia production from urease-positive bacteria. One additional sample had a urine pH <4.0 that was deemed implausible and therefore was also excluded.

Characteristics of this subcohort of 980 individuals are similar to the overall characteristics of the full CRIC Study cohort, as previously published, including a mean age of 58 years, a mean estimated glomerular filtration rate (eGFR) of 44 ml/min per 1.73 m^2, and a population including 43% females and 41% non-Hispanic whites, with 51% of participants with diabetes.[25] The mean ± SD net acid excretion was 33 ± 18 mEq/day (Supplementary Figure S1). The percentage of acid excreted as ammonium was 46% ± 17%. The total net acid excretion and the percentage of acid excreted as ammonium were lower with a lower eGFR (Figure 1a). Participants with diabetes had overall higher acid excretion, lower urine pH, and a lower percentage of acid excreted as ammonium compared with those without diabetes (each $P < 0.05$). Participants with diabetes consumed more dietary protein as estimated by a food frequency questionnaire ($P < 0.01$), which may, in part, explain their higher acid excretion.

Although net acid excretion should approximate nonvolatile acid load in steady state, this concept has been questioned in CKD, with some studies suggesting daily acid retention due to net acid excretion that is lower than estimated diet-dependent acid load.[22–24] Therefore, we evaluated the relationship between acid excretion and objective markers of acid production, including urine sulfate and citrate. Milliequivalents (mEq) of sulfate in the urine represent an equal amount of acids produced during the metabolism of organic sulfur in dietary protein. Similarly, mEq of urinary organic anion salts represent equal amounts of acids net produced by endogenous generation of organic acids in metabolic processes including the tricarboxylic acid cycle. Overall, urine citrate and sulfate were lower in participants with a lower eGFR (Figure 1b; each $P < 0.01$ from a continuous linear model), but both were higher in participants with diabetes independent of the eGFR (each $P < 0.05$).

Clinical determinants of acid excretion in CKD

Clinical characteristics according to quartiles of net acid excretion are reported in Table 1. In these univariate analyses, higher net acid excretion was associated with male sex, white race, greater body size, greater eGFR, and lower serum potassium, among other variables (Table 1). Consistent with the effect of diet on nonvolatile acid load, higher quartiles of net acid excretion were also associated with higher caloric intake and higher estimates of acid load from dietary data (each $P < 0.01$; Table 1). After standardization to a 2000-kcal diet, higher acid excretion was associated with a greater relative intake of animal proteins, which are known sources of acid, and a lower relative intake of fruits and vegetables, which are known sources of alkali (Table 1).

Although not associated in univariate models, in multivariable models that account for demographic characteristics, diabetes, and eGFR, higher net acid excretion was associated with lower serum bicarbonate (0.17 mEq/l lower serum bicarbonate per 10 mEq/day higher net acid excretion; $P < 0.01$), consistent with a larger acid load. Relationships were similar in those with and without diabetes (data not shown). This result was similar if additionally adjusted for